IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	
Ludviksson *et al.*	
Serial No. 09/856,544	Group Art Unit: 1644
Filed: December 20, 2001	Examiner: Haddad, M.
FOR: ANTAGONISTS OF THE αEβ7 INTEGRIN AS THERAPEUTIC AGENTS FOR INFLAMMATORY DISEASES	Confirmation No. 9811

DECLARATION OF EUGENE C. BUTCHER UNDER 37 C.F.R. § 1.132

Commissioner for
Patents
Washington, D.C. 20231

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Sir:

I, Eugene C. Butcher, a citizen of the United States of America, residing at 230 Corte Madera, Portola Valley, CA 94028, declare that:

1. I have read and understood the above-referenced patent application.

2. I received my M.D. degree from Washington University in St. Louis, Missouri. I have been conducting research in the field of inflammation since 1978 and am a co-author of over 200 publications relating to inflammation. I am currently a Professor in the Department of Pathology at Stanford University and a co-founder of Bioseek, Inc.

3. It is my belief that although U.S. Patent No. 5,610,281 (the '281 patent) discloses the administration of an isolated peptide derived from the extracellular domain of E-cadherin, which

binds to αEβ7 and inhibits the adhesion between an IEL and E-cadherin, this interaction between αEβ7 and E-cadherin is not relevant to the present claims, because E-cadherin is expressed in epithelial cells and is not expressed to any appreciable extent in non-epithelial cells of the gut, such as the lamina propia, where inflammatory bowel disease manifests itself (Strauch et al. "Integrin αEβ7 Mediates Adhesion to Intestinal Microvascular Endothelial Cell Lines Via an E-Cadherin-Independent Interaction" *J. Immunol.* 166: 3506-3514, 2001; Hanby et al. "Downregulation of E-Cadherin in the Reparative Epithelium of the Human Gastrointestinal Tract" *Am. J. Pathol.* 148:723-9, 1996; Cepek et al. "Integrin αEβ7 mediates adhesion of T-lymphocytes to epithelial cells" *J. Immunol.* 150:3459, 1993; Cepek et al. "Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the αEβ7 integrin" *Nature* 372:190, 1994; Karcela et al. "Recognition of E-cadherin on epithelial cells by the mucosal T cell integrin αM290β7 (αEβ7)" *Eur. J. Immunol.* 25:852, 1995; Higgins et al. "Direct and regulated interaction of integrin αEβ7 with E-cadherin" *J. Cell Biol.* 140:197, 1998). Therefore, the suggestion that the interaction between αEβ7 and E-cadherin can be inhibited to treat inflammatory bowel disease is not supported by the longstanding understanding of E-cadherin expression and mechanisms of inflammatory bowel disease. My belief is based on the following additional scientific facts and observations.

Although E-cadherin has been identified as an αEβ7 ligand, this is not the only ligand for αEβ7. αEβ7, like other integrins, binds to more than one ligand. For example, the I-domain containing integrin, aMb2(Mac 1), binds a wide repertoire of ligands, including ICAM-1, fibrinogen, iC3b, factor X, denatured proteins, neutrophil inhibitory factor, lipopolysaccahride and zymosan. Yalamanchili et al. (*J. Biol. Chem.* 275: 21377, 2000) have shown that the I-domain is required for binding to some ligands and not others. Similarly, another integrin, α4β7, has multiple ligands, including MadCAM-1, VCAM-1 and fibronectin. Monoclonal antibodies have been generated that define unique patterns of inhibition for α4β7 binding to each of its defined molecular ligands (Andrew DP, *J. Immunol.* 153:3847, 1994). Yet another example is the α3β1 integrin which binds collagen, laminin and fibronectin. However, only the binding of α3β1 integrin to fibronectin, not the binding of α3β1 integrin to laminin or collagen, can be

inhibited by RGD-containing peptides (Elices, MJ, *J. Cell Biol.* 112:169, 1991). The αEβ7 integrin is distinct from other integrins in containing both an I-domain and an X domain of 55 amino acids. Binding of alphaE to E-cadherin does not appear to involve the X domain and it is believed that the X domain may be involved in interactions with yet unidentified αEβ7 ligands (Higgins et al. J. Biol. Chem. 275:25662, 2000). Therefore, it is well accepted that an inhibitor of a ligand for a particular integrin is not expected to be an inhibitor of all of the ligands which bind to that integrin.

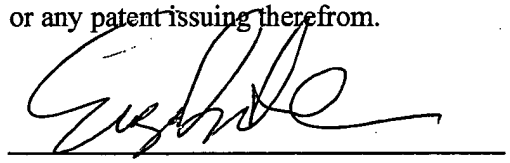
As stated above, it is also known that E-cadherin is not expressed to any appreciable extent in the lamina propia of the gut. Therefore, it is highly unlikely that inflammatory bowel disease is mediated via an αEβ7-E-cadherin interaction. The publication by Strauch et al. ("Integrin alpha E(CD103)beta 7 mediates adhesion to intestinal microvascular endothelial cell lines (HIMEC) via an E-cadherin-independent interaction," *J. Immun.* 166: 3506-3514, 2001) reports that αEβ7 mediates adhesion to intestinal endothelial cells in the lamina propia *in vivo*, via an E-cadherin-independent interaction. Strauch et. al. also suggest the presence of a second ligand for αEβ7 that is clearly distinct from E-cadherin and with a different expression pattern. This reference also showed that a mAb that blocks E-cadherin mediated cell binding to αEβ7, did not block binding of HIMEC to an αEβ7-Fc. Furthermore, HIMEC did not express detectable levels of E-cadherin.

Another reference has suggested that αEβ7 mediates lymphocyte binding to a skin-derived epithelial cell line through an E-cadherin independent interaction (Brown et al. "Mechanisms of binding of cutaneous lymphocyte-associated antigen-positive and alphaEbeta7-positive lymphocytes to oral and skin keratinocytes" *Immunology* 98:9-15, 1999). Further evidence that αEβ7 can have a different, E-cadherin independent role in cell homing and inflammation comes from gene expression analysis showing that αEβ7 is expressed on T cell populations outside the intraepithelial area, e.g. in areas that do not contain E-cadherin expressing cells (Gavin M. et al., "Homeostasis and anergy of CD4(+)CD25(+) suppressor T cells in vivo" *Nat. Immunol.*, 3(1):33, 2002; Zelenika D., "Regulatory T cells overexpress a subset of Th2 gene transcripts" *J. Immunol.* 168(3): 1069, 2002; McHugh RS et al.,

"CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor" *Immunity* 16(2):311, 2002).

Therefore, it would not be expected that the identification of an inhibitor of E-cadherin binding to αEβ7, would provide an inhibitor for the interaction of αEβ7 with another ligand, such as the ligand involved in inflammatory bowel disease. Although the '281 patent discloses that administration of an E-cadherin peptide leads to decreased localization of intra-epithelial lymphocytes to the intraepithelial compartment, the '281 patent does not show or suggest that an αEβ7 Mab will inhibit binding of αEβ7 to another ligand or that an αEβ7 Mab will inhibit any activity associated with the interaction of αEβ7 with a non-E cadherin ligand. Furthermore, because E-cadherin is not found at appreciable levels in the lamina propia, it would not be likely that an E-cadherin based mechanism would be successful for treatment of inflammatory bowel disease, and it would not have been obvious, at the time the present invention was made, to target an E-cadherin ligand, e.g. αEβ7, to treat inflammatory bowel disease.

4. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any patent issuing therefrom.



EUGENE C. BUTCHER

2/25/03

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